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AZADIRACHTIN EFFICACY IN COLORADO POTATO BEETLE AND WESTERN FLOWER THRIPS CONTROL

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ABSTRACT

Colorado potato beetle (CPB) and western flower thrips (WFT) are agricultural pests which are difficult to control due to resistance developed to many classes of insecticides. A botanical insecticide azadirachtin could represent an ecologically acceptable alternative for their management. This research tested the efficacy of azadirachtin on CPB and WFT mortality and feeding intensity, as well as thrips oviposition. Azadirachtin was used in three doses in laboratory trials. The results showed that this botanical insecticide was effective on larval stages of CPB and reduced the damages on treated leaves, but did not cause significant mortality or reduction in feeding intensity of the adults. It was not sufficiently effective against the WFT adults either, but it reduced the feeding damage on leaves and prevented egg laying. The results indicated that, even though azadirachtin did not have a significant adverse effect on the adult CPB and WFT insects, it reduced the overall population by affecting the CPB larvae or feeding intensity and egg laying in WFT. Antifeedant and anti-ovipositional activities, as shown in the case of WFT, should also be considered when assessing azadirachtin efficacy on insect pests.

Keywords: azadirachtin, *Frankliniella occidentalis, Leptinotarsa decemlineata*, mortality, neem.

INTRODUCTION

Botanical insecticides are natural compounds with insecticidal properties and their use in crop protection is as old as agricultural practice. Although they have been used for hundreds of years, the invention of synthetic insecticides has mostly displaced their use today. Due to fast action, low cost, easy application and high efficacy against a wide range of pests, synthetic insecticides have become an important part of pest management in modern agriculture (Korunić and Rozman, 2012). However, after decades of use, numerous negative side effects have been reported, including the development of insect resistance, pesticide residues in food, environmental pollution, toxicity to non-target organisms and negative impact on human health (Igrc Barčić and Maceljski, 2001). Negative side effects of synthetic insecticides have led to the investigation of various measures, ecologically more favorable than conventional pest management practices, and the development and use of natural plant protection agents. Many plant secondary metabolites have insecticidal, repellent, antifeedant or growth disrupting properties, making them potential candidates for use in pest control (Igrc Barčić and Maceljski, 2001). Many of them pose a minimum negative risks on human health, beneficials and the environment (Isman, 2006; Pavela, 2007; Kovaříková and Pavela, 2019).

Botanical insecticide neem is obtained from the neem tree *Azadirachta indica* A. Juss. It has a great potential as a natural pesticide because it contains several biologically active substances called triterpenoids, such as azadirachtin, nimbin, salinnine, nimbidin, gedunin etc. (Morgan, 2008). The main active ingredient is azadirachtin which acts against a wide range of insects that feed by biting and chewing or sucking on the leaves. It is ecotoxicologically acceptable and not harmful for beneficial organisms and humans

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(Grdiša and Gršić, 2013). Besides insecticidal, azadirachtin exhibits repellent, antifeedant, insect growth regulatory and anti-ovipositional properties (Schmutterer, 1990). The pests do not necessarily die immediately after ingestion; first they stop feeding on plants, and mortality can occur later as a result of no feeding. Repellent, antifeedant and anti-ovipositional actions of azadirachtin can be considered as insecticide's secondary effects, since they do not cause pest mortality directly, but they result in reducing the damage or pest population numbers (Kivan, 2005). These properties should be taken into account when evaluating the overall insecticidal effect.

Environmental Protection Agency placed this botanical in the 4th group according to toxicity (EPA, 2017). It is not persistent in the environment as it rapidly degrades under the sunlight (Boursier et al., 2011). It is not volatile, so insects cannot smell it. The median lethal oral dose (LD_{50}) for rats is >5000 mg/kg (Korunić and Rozman, 2012). In addition to being more environmentally friendly, the occurrence of neem resistance is highly unlikely since neem consists of several chemical compounds, while a large number of synthetic insecticides are composed of only one chemical compound, resulting in much greater possibility of developing resistance (Natureneem, 2017). Today, neem substances are sold under commercial names such as NeemGold, NeemAzal, Econeem, Neemark, Neemcure and Azatin in many countries, e.g. the United States, India, Germany and some Latin American countries (Silva-Aguayo, 2017). In Croatia, until recently, the only registered azadirachtin based preparation was NeemAzal-T/S, whose temporary registration was abolished in November 2018.

Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say 1824 and western flower thrips (WFT) *Frankliniella occidentalis* Pergande, 1895, are economically important pests which developed resistance to many classic chemical insecticides and became difficult to control (Maceljski and Igrc Barčić, 1992; Maceljski, 1995a; 1995b; Šimala, 2002). For Colorado potato beetle management in Croatia many insecticides are available (Bažok, 2020), but they belong mostly to organophosphates and pyrethroids to which the resistance was detected in central Croatia, the main potato production area (Bezjak et al., 2006). Western flower thrips is a polyphagous pest that has many generations per year, so frequent chemical control also led to development of pesticide resistance (Šimala, 2002). This research aimed to determine the efficacy of azadirachtin as ecologically more favorable solution for these pests' management. The effect on mortality and feeding of different Colorado potato beetle development stages, as well as on mortality, feeding and oviposition of western flower thrips adults were tested.

MATERIAL AND METHODS

In laboratory trials with both Colorado potato beetle and western flower thrips, preparation NeemAzal-T/S, manufactured by Pro-eco, was used. The formulation was emulsion concentrate (EC), with azadirachtin as active substance in a concentration of 10 g/l. According to the manufacturer's instructions (Pro-eco, 2018), it can be used on a large number of cultural crops such as vegetables, citrus fruits, grape vines, pome and stone fruit (except pears), raspberries, blackberries, blueberries, currants, strawberries, olives, figs. It is used for the control of aphids, whiteflies, leaf miner flies, owlet moths, thrips, Colorado potato beetle, cabbage root fly, moths, citrus leafminer, European grape vine moths, cicadas, winter moth, citrus flatid planthopper, olive moth, true bugs etc. The recommended dose is 3 l/ha (Pro-eco, 2018).

Colorado potato beetle trials

Adult beetles were collected at the end of May 2016 in Bjelovar-Bilogora County and reared in order to obtain the larvae. Rearing was done for several days in entomological cages provided with fresh untreated potato leaves. Laid eggs were separated regularly to another cage to avoid being eaten by the adults. In laboratory trials, first (L1) and third (L3) larval developmental stages were used.

The adults tested in trials were collected at the beginning of June 2016 on the same field locations. The experiments for both larvae and adults included four variants (three doses of NeemAzal-T/S and untreated control) (Table 1) and were preformed according to the IRAC method No. 7 (FRAC/IRAC, 1990). Every variant in trials with larvae was set up in four repetitions and included ten larvae per repetition, while variants in trials with the adults included ten repetitions with one adult per repetition. Mortality was recorded every 24 hours for four days or until all individuals in the experiment died. The efficacy of azadirachtin on all variants including control was calculated according to Schneider-Orelli (1947):

$$Efficacy\ (\%) = \frac{mortality\ on\ treatment\ (\%) - mortality\ on\ control\ (\%)}{100 - mortality\ on\ control\ (\%)}\ x\ 100\%$$

For determining the effect of azadirachtin on feeding intensity, area of each potato leaf used in the trials with larvae and adults was measured on millimetre paper before the treatments. Every 24 hours for four days the measurements were repeated on the same paper and the differences in leaf area were recorded. If the estimated damage was higher than 80%, the leaf was replaced with a new premeasured one. Consumed leaf area in each variant (replication) was the basis for calculating the efficacy of azadirachtin in reducing the feeding intensity of larvae and adults. Efficacy was calculated according to the Abbott formula (1925):

$$Efficacy\ (\%) = \frac{consumed\ leaf\ area\ on\ control\ (mm) - consumed\ leaf\ area\ on\ treatment\ (mm)}{consumed\ leaf\ area\ on\ control\ (mm)}\ x\ 100\%$$

Western flower thrips trials

Before the experiment, western flower thrips were reared for two months on leek in glass jars in a climate chamber (24 ± 1°C, 50-60% RH, and 18:6 h [L:D] photoperiod). To obtain uniformly aged adults, four days before the experiment pupae were separated and put on untreated bean leaves to develop in the chamber into the adult stage. Bean seedlings (*Phaesolus vulgaris* L.) needed for the experiment were seeded in four containers (75 cm x 20 cm x 15 cm) and watered regularly for 20 days. Two days before the trial started, the plants in containers were treated by watering the soil around the plants with different doses of azadirachtin. The experiment included four variants (three doses of NeemAzal-T/S and untreated control) (Table 1), each set up in four repetitions. On the day of the experiment treated bean leaves were placed in petri dishes on 1% agar and on each leaf 10 adult thrips were added. Mortality, leaf damage and oviposition were recorded every 24 hours for three days or until all individuals died. Damage on leaves was calculated by counting specific thrips damages (yellowish spots) under the microscope and expressed as the percentage of area where dechlorophyllation was observed. Azadirachtin efficacy was calculated in the same way as for the Colorado potato beetle.

Table 1. Doses and concentrations of azadirachtin used in experiments with Colorado potato beetle and western flower thrips in 2016

	Dose (l/ha)	Dose (ml/l)	Concentration (%)
Colorado potato beetle	2.00	0.20	2.00
	3.00	0.30	3.00
	4.00	0.40	4.00
Western flower thrips	1.50	0.15	1.50
	3.00	0.30	3.00
	4.50	0.45	4.50

Statistical Analysis

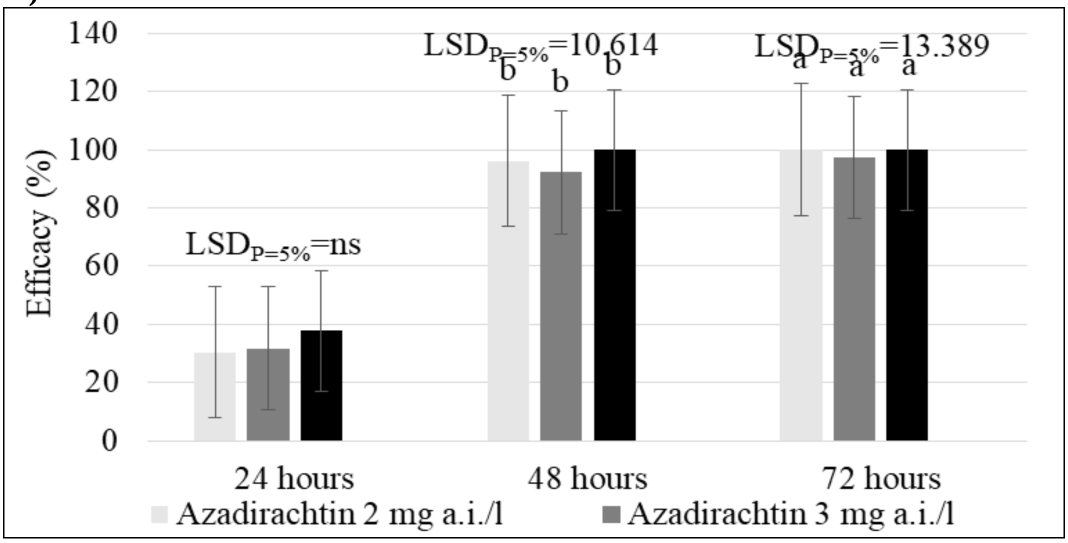
Data on efficacy and leaf damage were statistically evaluated by ANOVA and ranked using the Duncan's new multiple range test. The analysis was done by using statistical program ARM 9® (Gylling Data Management, 2015). The calculation of efficacy was only applied to those variants and dates for which a statistically justified difference was found between untreated control and variants in the trials. In case of an uneven data distribution, data were transformed with *log* (x + 1) transformation.

RESULTS AND DISCUSSION

Colorado potato beetle trials

In experiment with the adults, dead individuals were found in a very small number. Their morality varied between 10% and 30% on both treated variants and untreated control. Therefore, the efficacy of azadirachtin on the adults was not calculated. The results of the efficacy of various doses of azadirachtin on larvae are shown in Figure 1.

a)



b)

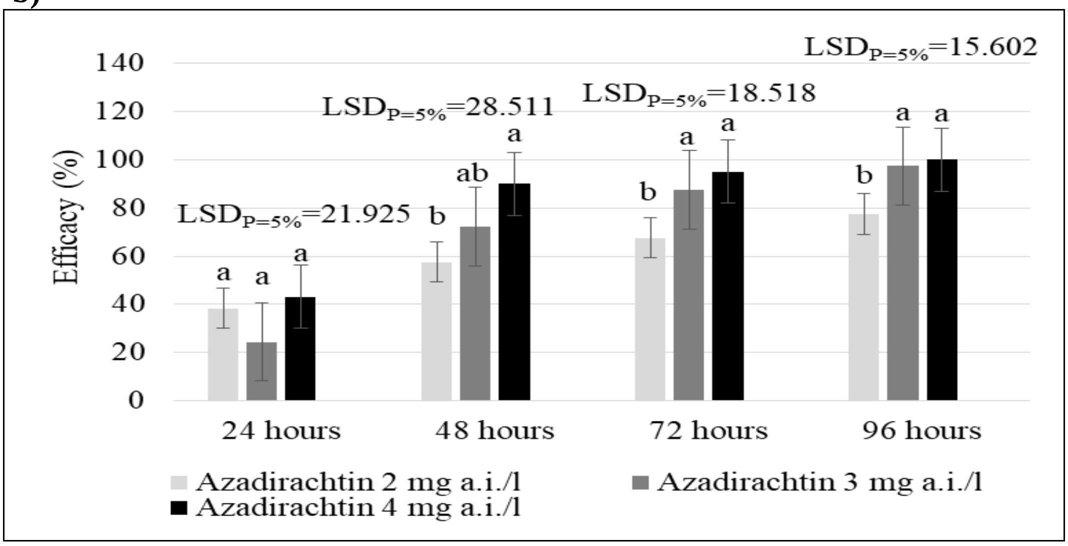


Figure 1. The efficacy (±SEM) of azadirachtin on Colorado potato beetle: a) L1 larvae; b) L3 larvae. Means followed by the same letter in particular group (hours) do not differ significantly (P = 0.05)

The average leaf area (mm^2) consumed by Colorado potato beetle L1 larvae, L3 larvae and the adults is shown in Figure 2.

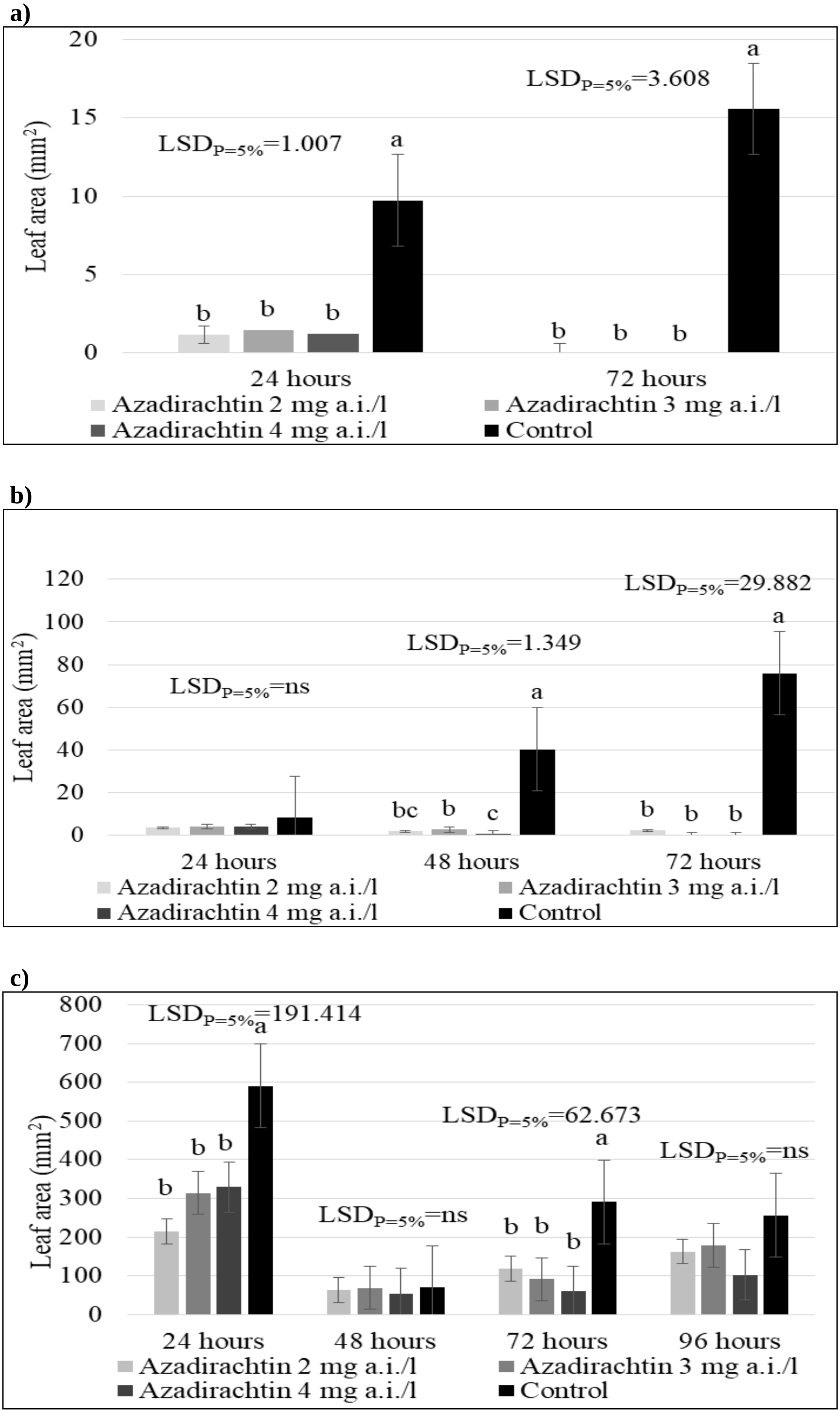


Figure 2. The average leaf area (mm^2) consumed by: a) L1 larvae; b) L3 larvae; c) adult beetles. Means followed by the same letter in particular group (hours) do not differ significantly (P = 0.05)

Azadirachtin efficacy on Colorado potato beetle L1 larvae (Figure 1a) was relatively fast and ranged from 25.66% to 33.70% after 24 hours, depending on the dose. After 48 hours, efficacy for all applied doses was greater than 90%, which can be considered satisfactory. After 72 hours, the overall efficacy was higher than 97.22%. Because of such high mortality, the L1 larvae caused significantly less damages on potato leaves when compared to control (Figure 2a).

Slightly higher efficacy (38.38% to 43.04%) was achieved on CPB L3 larvae 24 hours after the treatment (Figure 1b). This could be explained by the greater gluttony of L3 larval stage, as larval gluttony increases with a higher developmental stage (Maceljski, 2002). After 48 hours, the efficacy of all three doses of azadirachtin increased and ranged from 52.77% to 88.88%. Compared to L1 larvae, this is somewhat lower efficacy, which indicates greater sensitivity of younger larvae (Bezjak et al., 2006; Bažok et al., 2008). On the third day, azadirachtin achieved 62.85% to 94.28% efficacy, which increased with increasing doses. At the same time, larvae consumed considerably less leaf area (Figure 2b) compared to untreated control, with no significant differences between insecticidal variants. This indicates that for the repelling effect of azadirachtin, the dose of insecticide was not crucial.

Kovaríková and Pavela (2019) observed that CPB larvae treated with neem oil showed a reduction in food intake, which resulted in less leaf damage compared to control variant. The efficacy of azadirachtin on L1 and L3 larvae achieved in the performed experiments was considerably higher than that found in field trials done by Bezjak et al. (2006), and in laboratory experiments performed by Bažok et al. (2008). In both experiments, preparations of Celaflor Neem and Neem extract were used, so it is possible that the difference in efficacy was caused by the different contents of the extract in the formulations. The efficacy of Celaflor Neem in the field trials conducted by Bezjak et al. (2006) reached between 55% and 88% on the third day after the treatment, and after it was reduced presumably due to field conditions. The increased content of the active substance, as well as the improved formulations, can significantly contribute to a better effect of the agent. Also, under field conditions, the application of the same insecticide can result in lower efficacy compared to the efficacy achieved in the laboratory experiments. Ropek and Kołodziejczyk (2019) in their field study showed that the level of potato yields on the plants protected by Neem extract, was lower than on the chemically protected plants, but the differences were not statistically significant, which points to a considerable yield protective role of neem extract in potato cultivation.

In our experiments, azadirachtin did not obtain satisfactory efficacy on the adults of Colorado potato beetle and their mortality was not significantly different of that found in control variant. Such results could be explained by insecticide’s repelling action, which causes the adults to feed less on treated leaves and not ingest the insecticide in quantities that cause mortality. Also, these beetles can withstand a longer periods of time without food (Maceljski, 2002), allowing them again not to come in contact with the insecticide. Although azadirachtin did not cause high mortality, it reduced the leaf damage done by CPB adults. However, this effect was not so pronounced in the adults as it was the case with both L1 and L3 larvae.

Western flower thrips trials

The efficacy of azadirachtin against adults of western flower thrips is shown in Figure 3.

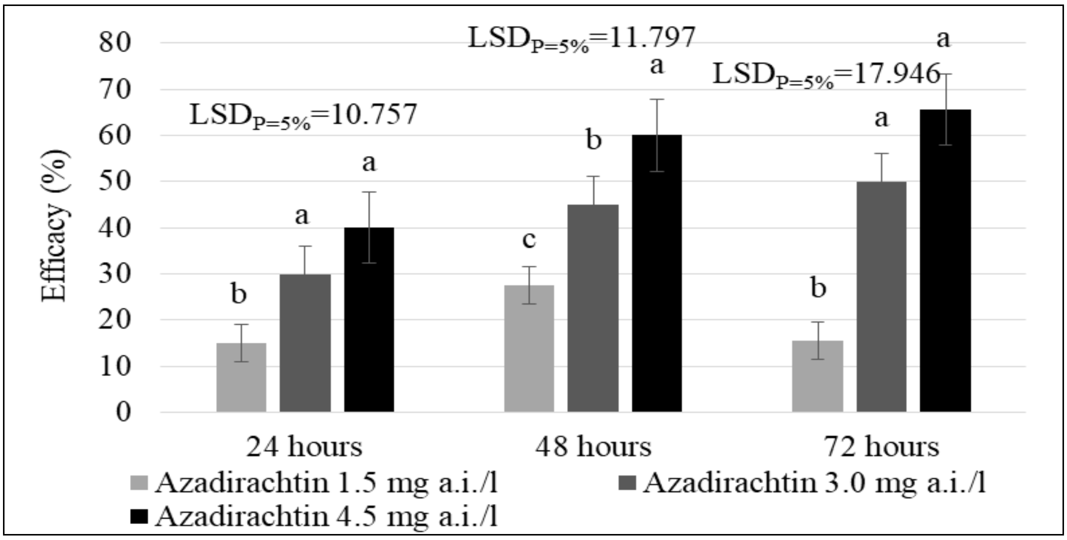


Figure 3. The efficacy of azadirachtin on western flower thrips adults.
Means followed by the same letter in particular group (hours) do not differ significantly (P = 0.05)

The efficacy of azadirachtin on western flower thrips adults during 72 hours depended on the applied dose (Figure 3). The efficacy of all three doses after 24 hours ranged from 15% to 40%. After 48 hours and 72 hours, the highest efficacy was achieved by the highest dose applied, and it ranged from 60% to 65%, respectively. This slow action and insufficient effect on WFT mortality cannot be considered satisfactory. Since azadirachtin effect in case of WFT was tested by watering the soil and not the foliage, the achieved efficacy is presumably the result of systemic insecticide activity. It is likely that the initial effectiveness would be better with foliar application. Thoeming et al. (2003) investigated the systematic effect of azadirachtin on WFT larvae, by using azadirachtin in doses of 2.5 mg/l, 5 mg/l and 10 mg/l. Similarly, after 96 hours the highest dose achieved larval mortality of 50.6%. Even though they have used different doses and tested the larval stage, both experiments show that azadirachtin has a certain systemic effect on reducing the WFT population. In order to be effective, azadirachtin must be ingested by pests, which promptly leads to termination in feeding (usually within 24 hours), but the insect mortality does not occur immediately. In this trial, the efficacy was recorded for only three consecutive days after the application, so we cannot say for sure whether the efficacy after a longer period of time would be higher. In addition, azadirachtin acts as an insect growth regulator, affecting the developmental (larval) stages. This study was conducted on adult forms of thrips, and this may also be one of the reasons why azadirachtin did not achieve a satisfactory efficacy on WFT adults. Damages of western flower thrips on bean leaves are showed in Figure 4.

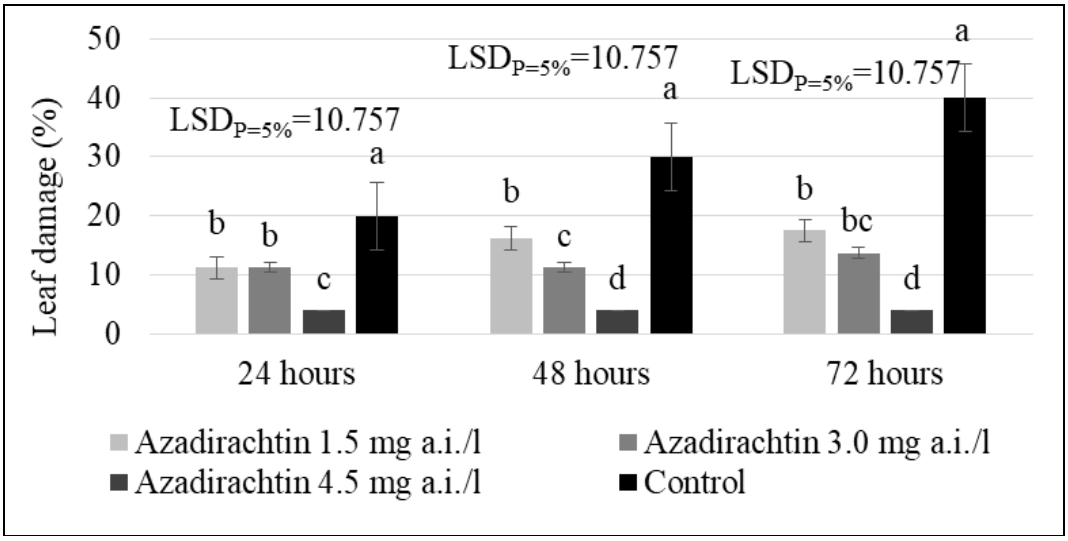


Figure 4. Observed damages caused by western flower thrips adults on different treatments in the experiment. Means followed by the same letter in particular group (hours) do not differ significantly (P = 0.05)

Compared to untreated control, all variants in which azadirachtin were used showed a significant reduction in WFT damage on bean leaves. The highest dose had the best result during all three days, reducing the damages to only 4%. The other two doses did not achieve such a good efficacy, but damages were still significantly lower than the ones detected on control variant. Effect of azadirachtin on WFT oviposition is showed in Table 2.

Table 2. Oviposition of western flower thrips in different treatments in experiment with azadirachtin efficacy

	mg a.i./l	The number of repetitions on which oviposition was detected		
		24 hours	48 hours	72 hours
Azadirachtin	1.5	2	4	4
	3.0	1	2	3
	4.5	0	0	0
Untreated control	0.0	4	4	4

Azadirachtin proved to be effective in preventing the oviposition, but this depended on the dose. When used at a maximum dose of 4.5 mg a.i./l, no eggs were found during the whole experiment. The recommended dose (3.0 mg a.i./l) showed somewhat weaker effect, while the lowest dose had no anti-ovipositional effect and was not different than the control. On untreated control eggs were found in all four repetitions and in every time phase of the experiment. Azadirachtin acts on insects in such way that they stop copulating and depositing eggs, and if eggs are laid, most of them are sterile (Kivan, 2005). These results confirmed the complete cessation of oviposition at the highest dose applied. It would be interesting to investigate are there any differences in plant varieties on which eggs are laid in case of recommended or lower doses, as well as the eggs' sterility.

CONCLUSIONS

Azadirachtin proved to be efficient against Colorado potato beetle larval stages, causing high mortalities and significantly reducing the damages on potato leaves. It did not have such good effect on the adults. However, the suppression of larval stages leads to reduction of the overall adult population of Colorado potato beetles and consequently, to lesser damages done by the adults. Also, larvae feeding less can result in poorer shape of the adults that develop from such larvae, and this may have a significant influence on their biological potential. These assumptions, as well as the biotic potential of beetles that develop after ingesting azadirachtin either in the larval stage or as an adult, should be further investigated. In the case of western flower thrips adults, azadirachtin was not highly effective and caused 50% mortality after three days. So, it could still be used to initially lower the population numbers or in combination with other insecticides or biological agents, especially as this is an environmentally friendly insecticide. Higher doses also reduced the oviposition, which can prevent further population growth. Since these higher doses are not recommended, further research should be conducted in order to determine whether the recommended dose could have such effect after longer period of time. When evaluating the biological plant protection products, it is important to consider the secondary effects of these products, such as the reduction of feeding or oviposition, because they contribute to the insecticide overall effect on decreasing the pest population and reducing the damages.

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